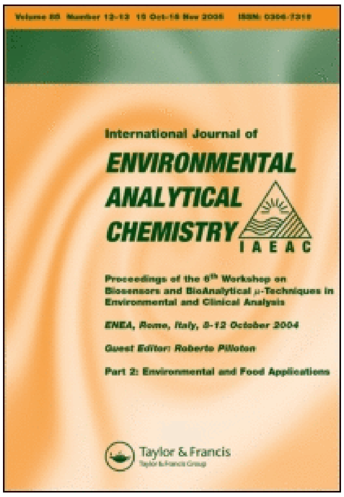

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Suitability of Different ^{13}C Solid-state NMR Techniques in the Characterization of Humic Acids

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SUITABILITY OF DIFFERENT ^{13}C SOLID-STATE NMR TECHNIQUES IN THE CHARACTERIZATION OF HUMIC ACIDS

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Qualitative and quantitative analyses of humic acids (HAs) with five different ^{13}C solid-state NMR techniques were assessed using HAs of various origins and locations. The NMR techniques compared are: (1) direct polarization/magic angle spinning (DP/MAS) at 13 kHz, (2) conventional cross polarization (CP)/MAS at 5 kHz, (3) ramp-CP/MAS at 8 kHz, (4) CP/total sideband suppression (TOSS) at 4.5 kHz, and (5) DP/MAS corrected by CP/spin-lattice relaxation with TOSS. The spectra from the five techniques were first compared qualitatively. Then, each spectrum was divided into eight regions for quantitative evaluation. DP/MAS spectra were used as quantitative references. Ramp-CP/MAS and CP/TOSS spectra gave consistently better results than those of the conventional CP/MAS spectra at a ^{13}C frequency of 75 MHz, which were incorrect due to spinning sidebands. CP/MAS at low magnetic fields (22.6 and 50.6 MHz ^{13}C frequency) indicated improved integration results but lower resolution. Correction factors calculated by comparison with DP/MAS will be useful to convert the non-quantitative peak areas in the CP/TOSS and ramp-CP/MAS spectra into more quantitative results.

Keywords: Solid-state NMR; Quantitative ^{13}C NMR; Nuclear magnetic resonance; Cross-polarization (CP) magic-angle spinning (MAS) NMR; Structure of humic acids

INTRODUCTION

Humic acids (HAs) play important roles in many agricultural and environmental reactions. Information on their structure is critical for understanding their reactivity with organic and inorganic contaminants [1–6]. However, due to its complexity and heterogeneity, it has proved difficult to determine a HA structure. Classical elemental analysis data and chemical degradation techniques provide only limited information on

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the possible chemical constituents and building blocks of HAs [7,8]. Recently, many spectroscopic methods such as infrared, electron spin resonance, Raman, fluorescence and NMR have been used to investigate HA compositions and structures [9]. Among those, nuclear magnetic resonance spectroscopy (NMR) is one of the most powerful tools [10,11].

Solid-state ^{13}C direct polarization/magic angle spinning (DP/MAS) ^{13}C NMR has been used for studying organic molecules for two decades. However, it requires that the recycle delay between scans must be five times longer than the longest T_1^C (^{13}C spin-lattice relaxation time) in a sample to obtain a quantitative ^{13}C NMR spectrum. The longest T_1^C's range from 5 s to over 10,000 s in most crystalline or organic solids. We have found crystalline aliphatic long $-(CH_2)_n-$ in HAs [12], and their longest T_1^C's can be of the order of tens of seconds or even must longer [13]. Thus, very long recycle delays may have to be used to obtain quantitative DP/MAS spectra. Therefore, considering instrument time it may not be practical to use only DP/MAS to analyze HAs with long T_1^C's.

The most popular ^{13}C solid-state NMR technique in studying HA structures is cross polarization/magic angle spinning (CP/MAS) [14]. This technique improves sensitivity substantially through the transfer of magnetization from the abundant ^{1}H to dilute ^{13}C spins by cross-polarization (CP) [15]. However, CP/MAS spectra are not quantitative [11,13,14,16,17]. The first problem is the reduced CP efficiency for unprotonated carbons, mobile components, or regions having short $T_{1\rho}^H$ (proton rotating-frame spin-lattice relaxation time). The second is spinning sidebands, which reduce the intensity of the centerband, resulting in the loss of intensity and distortion of peak areas. Even more seriously, the sideband of a strong aromatic peak may overlap with a small aliphatic signal, and distort its intensity significantly. For instance, with a spinning speed of 5 kHz and a 200 MHz ^{1}H frequency, the ^{13}C sidebands are separated by 100 ppm [18–21]. As a result, the sidebands of the aromatic signals between 115 and 150 ppm overlap directly with the aliphatic signals between 15 and 50 ppm. The sideband intensities at these conditions are ca. 12% of the aromatic centerband, so that the small aliphatic signals in highly aromatic samples are seriously overestimated [18–21].

The third is the baseline distortion due to a deadtime associated with each pulse. The extensive application of CP/MAS technique in the literature has led to the underestimation of the sp^2-carbon region and overestimation of the sp^3-hybridized carbons in humic substances. With the availability of higher-field spectrometers, the problem of spinning sidebands, in particular for sp^2-hybridized carbons with their large chemical-shift anisotropies, has become more severe; the literature contains strongly distorted spectra with many sidebands acquired at low spinning speed and a magnetic field strength equivalent to a ^{1}H frequency of 400 MHz.

The total sideband suppression (TOSS) pulse sequence, applied before the start of signal detection, was developed to remove spinning sidebands [22,23]. Nevertheless, at low spinning speeds, even under the most ideal conditions, CP/TOSS spectra are not completely quantitative because the intensity of the suppressed sidebands is not fully added to the centerband. Aromatic and carbonyl groups are underestimated because their sidebands are big due to their large chemical-shift anisotropy [24,25]. Furthermore, transverse ^{13}C T_2^C relaxation (spin–spin relaxation) occurs during the implementation of TOSS, which might lead to a differential decrease in signal intensities [26]. We have not seen many TOSS spectra of humic substances in the literature up to now, even though high-speed CP/TOSS with good Hartman–Hahn match can yield very good semi-quantitative results.

It had been thought that high-speed MAS, while reducing the sidebands, also interferes with the CP efficiency [15]. Thus, CP/MAS experiments are often run at a spinning speed below 5 kHz. However, at this speed, significant sidebands can occur. In order to overcome this dilemma, ramp-CP/MAS was developed to establish an efficient Hartmann–Hahn matching condition at a relatively high MAS speed [27–29]. Ramp-CP/MAS was recently applied in studying humic substances [30,31]. This techniques is also non-quantitative because the relatively long contact times needed render some carbon species with short $T_{1\rho}^{\mathrm{H}}$ in humic substances invisible. Actually, it should be realized that efficient fixed-amplitude CP can also be used at high spinning speeds if the Hartman–Hahn match is adjusted to the sideband condition at the particular spinning frequency.

DP/MAS combined with T_1^{C} correction obtained from CP/T_1-TOSS spectra was first used to quantify polymer crystallinity [32]. We applied this technique to the quantitative characterization of HAs [33]. In this technique, direct polarization is employed and the reduced CP efficiency problem can be avoided. By employing high spinning, sidebands are reduced to an insignificant proportion. If HAs have long T_1^{C}'s, CP/T_1-TOSS spectra are used to correct for incomplete relaxation. For many of the samples chosen for this study, the correction factors are close to unity. DP/MAS has been shown to observe essentially all organic carbon present [20,21] and is therefore a reliable reference method.

The objective of this study is to assess the above five solid-state ^{13}C NMR techniques and to evaluate their limitations and advantages. Golchin *et al.* [34,35] compared the DP/MAS and CP/MAS spectra of a HF-treated whole soil and three density fractions and concluded that the proportions of aromatic and carbonyl carbons were lower and those of alkyl and *O*-alkyl were higher in CP/MAS. The previous studies do not include ramp-CP/MAS and CP/TOSS. Our study is more comprehensive using five NMR techniques and nine HAs of various origins and locations. We hope that this paper will provide a better understanding of solid-state ^{13}C NMR techniques and their application in characterizing humic substances and other amorphous organic materials.

EXPERIMENTAL

Origins and Preparations of HAs

Six soil HAs (from Germany, Amherst, New Hampshire, New York, Florida, and Minnesota) and three commercial HAs [Aldrich (Aldrich Chemical Company), IHSS (International Humic Substances Society) and ARC (ARCTECH, Inc.)] were used. Their origins, locations and elemental compositions [33] as well as the details of extraction and purification [8] were described elsewhere. Aldrich HA was re-extracted and purified in our laboratory before use.

NMR Spectroscopy

For DP/MAS, a HA sample was packed in a 4 mm-diameter zirconia rotor with a Kel-F cap. Samples were run at a ^{13}C frequency of 75 MHz in a Bruker DSX-300 spectrometer at a high spinning speed of 13 kHz. The ^{1}H 90° and ^{13}C 180° pulse lengths were 3 and 6 μs, respectively. In the Hahn spin echo, either a short period of 10 μs or one rotation period (77 μs) was employed as the pre-echo delay to avoid baseline distortions. The exact timing of the start of detection for undistorted spectra was determined on a ^{13}C-labeled model compound (a mixture of ^{13}C-labeled amino acids) with signals at both ends of ^{13}C chemical-shift range. This model compound was also used to determine optimum NMR parameters such as ^{1}H and ^{13}C 90° pulse length. The number of scans varied from 8192 to 32,768, depending on individual samples.

For conventional CP/MAS, the spectra obtained at a ^{13}C frequency of 75 MHz were run in a Bruker MSL-300 spectrometer at a spinning speed of 5 kHz. Samples were packed in a 7 mm-diameter zirconia rotor with a Kel-F cap. The ^{1}H 90° pulse length was 4.1 μs and ^{13}C 180° pulse length was 6.4 μs. The contact time was 1 ms. A Hahn echo was employed before detection in order to remove baseline distortion. The recycle delay was 1 s with 4096 or 8192 scans. The CP/MAS ^{13}C spectrum of Minnesota HA at a ^{13}C frequency of 22.6 MHz was run at a spinning speed of 4 kHz with a contact time of 1 ms and 10000 scans. The CP/MAS ^{13}C spectrum of IHSS HA at a ^{13}C frequency of 50.3 MHz was run with a contact time of 0.75 ms, 29000 scans, and a spinning speed of 5 kHz.

For CP/TOSS, samples were packed in a 7 mm-diameter zirconia rotor with a Kel-F cap and run at 75 MHz in a Bruker MSL-300 spectrometer at a spinning speed of 4.5 kHz. The ^{1}H 90° pulse length was 3.4 μs and the ^{13}C 180° pulse length was 6.4 μs. The contact time was 800 μs. The recycle delay was 1 s with 4096 scans.

Ramp-CP/MAS spectra were obtained on a Bruker DSX-300 spectrometer. Samples were packed in a 4 mm-diameter zirconia rotor with a Kel-F cap. The ramp covering 50–100% of the nominal Hartmann–Hahn match was applied to the carbon channel. The contact time was 2.5 ms and the recycle delay 1 s. The spinning speed was 8 kHz, with 8000–20000 scans.

CP/T$_1$−TOSS is a pulse sequence modified from CP/TOSS. After the contact time and before the π-pulse train, a $+z/-z$ filter was applied so that the signal decays from full intensity to zero as a result of T_1^{C} relaxation. This experiment not only estimated ^{13}C T_1 but also yielded the T_1 corrections for the various peaks in the incompletely-relaxed DP/MAS spectra, using two spectra at different filter times. One filter time was 0.5 ms and the other was equal to the recycle delay employed in DP/MAS [33]. Samples were packed in a 7 mm-diameter zirconia rotor with a Kel-F cap and run at 4.5 kHz and a ^{13}C frequency of 75 MHz in a Bruker MSL-300 spectrometer at a spinning speed of 4.5 kHz. The ^{1}H 90° pulse length was 3.4 μs and the ^{13}C 180° pulse length 6.4 μs. The contact time was 800 μs. The recycle delay was 1 s and 4096 scans were averaged. DP/MAS combined with CP/T$_1$−TOSS correction has been described in detail elsewhere [33].

NMR Background

DP/MAS, CP/MAS [13,25,26,36], ramp-CP/MAS [27–31], CP/TOSS [22,23] and DP/MAS corrected by CP/T$_1$−TOSS [32,33] have been discussed in detail elsewhere and will not be dealt with thoroughly here. But we will here address the Hahn echo before detection, which can be used to minimize baseline distortion often found in published spectra.

Hahn Echoes under MAS

Various published ^{13}C MAS spectra of humic substances appear to have severely distorted baselines, which invariably entail significant distortions of the spectra themselves; in particular, broad spectral features are reduced in intensity. These artifacts are usually due to the dead time at the start of signal detection, which arises from finite pulse ring-down and filter/receiver response times. It is well known that a Hahn spin echo [37] before detection, with pulse sequence τ – 180°-pulse – τ as shown in Fig. 1, can minimize such baseline distortions. At the end of the second τ period, all isochromats are refocused along their common direction as at the very start of the precession, forming an echo. This is possible because the isochromats that have precessed by the largest angle during the first τ period will also return fastest.

However, under MAS, the choice of τ is restricted. A 180° pulse near the middle of the rotation period dephases the magnetization instead of refocusing it [38]. An ideal echo requires that τ is a rotation period (or a multiple thereof). However, a problem arises if the time $2\tau = 2t_r$ before detection is so long that significant T_2 relaxation occurs. Fortunately, in the case of fast spinning, the rotation period t_r, which is the inverse of the rotation rate, is short enough that T_2 relaxation during $2\tau = 2t_r$ is negligible. We have tested that the T_2^C's of solid HA samples are usually on the order of a millisecond or more, permitting echo periods of $2\tau = 150\,\mu s$ or longer to be used without spectral distortions.

Alternatively, the echo time 2τ can be chosen very short. Then, the difference in the magnitude of the precession angle (phase) covered by a given isochromat in either of the two τ periods will be small. At low spinning speeds, a quasi-static approximation can be used, where it is considered that the rotor, and thus the segments and interaction tensors, change their orientation insignificantly during 2τ. This is the approach taken in the CP/MAS experiments described below. In the case of fast spinning, the 2τ periods must be very short ($< 20\,\mu s$). Then, it can be argued that the precession phases remain proportionally small and the MAS effects are of second order. In practice, spectra with $2\tau < 20\,\mu s$ and with $2\tau = 150\,\mu s$ were found to be indistinguishable.

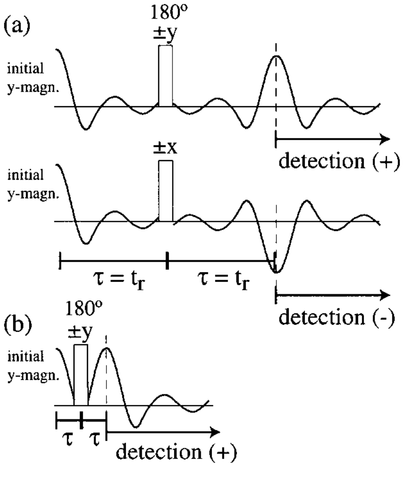

FIGURE 1 Pulse sequence for the Hahn echo, which permits dead-time free detection. Conditions for the delay τ under MAS are discussed in the text.

The optimum phase for the 180° pulse is different for the two approaches described. In the short-2τ case, the pulse phase should be along the direction of the initial magnetization; this minimizes errors due to deviations from the exact 180°-pulse length. In the case of the pulse being applied along the magnetization, the pulse length does not matter at all. On the other hand, for $2\tau = 2t_r$, the EXORCYCLE approach [39] should be used where the pulse phase is rotated through all quadrature directions $(x, y, -x, -y)$ with respect to the magnetization direction.

The exact timing of the start of detection should be determined using a sample with very good sensitivity, such as a ^{13}C-labeled model compound, that exhibits sharp peaks over the full spectral range (~30–180 ppm). For the 13 kHz DP/MAS spectra, timings that are off by as little as 2 μs can result in significant spectral distortions. Once the timings are set on the model compound, spectra of humic acids with excellent baselines are obtained using only constant phase correction (without linear/first-order correction).

RESULTS AND DISCUSSION

DP/MAS and Corrected DP/MAS

The elemental compositions calculated from both corrected DP/MAS and DP/MAS spectra were consistent with those from an elemental analyzer [33]. These results indicated that like DP/MAS, corrected DP/MAS is also a reliable technique for quantitative characterization of HAs.

The advantage of DP/MAS is that this technique uses direct polarization and the problems associated with CP are avoided. Furthermore, DP/MAS permits high spinning speed so that the sidebands are placed outside the region of the centerbands. Thus, we can easily integrate sidebands and add them to the centerbands. In our case, at a spinning speed of 13 kHz, the total sidebands of the aromatic group are reduced to less than 8% of the centerband. In addition, with the introduction of a rotation-synchronized Hahn echo, the baseline distortion can be essentially eliminated.

However, without CP the sensitivity of the DP experiments is greatly reduced [40]. Therefore, many more scans are needed to obtain a spectrum with good signal-to-noise ratio. In our experiments, it took just 4096–8192 scans for CP and 8192 to over 30000 scans for DP to obtain good spectra. Furthermore, for a quantitative DP/MAS spectrum, the relaxation delay should be five times longer than the longest T_1^C in a sample. The T_1^C's in HA samples vary widely. The experimental estimation of ^{13}C T_1^C relaxation times showed that ARC, IHSS, Florida, and New York HAs have short relaxation times (< 3 s); Amherst, Aldrich, Minnesota and New Hampshire HAs exhibit intermediate relaxation times (3–5 s); and German HA shows a very long ^{13}C T_1^C (~15 s). It is possible to obtain a quantitative spectrum for those HAs with short T_1^C's using DP/MAS alone. For HAs with intermediate and very long T_1^C's, it is extremely time-consuming to obtain a quantitative DP/MAS spectrum and the CP/T_1–TOSS correction was employed [33].

Comparison between Different Methods

The assignment of HA functional groups based on different chemical shift ranges is as follows [8,33]: 0–50 ppm, aliphatic groups; 50–60 ppm, methoxy groups and CαH in amino acids; 60–96 ppm, carbohydrate groups; 96–108 ppm, anomeric groups; 108–145 ppm, aromatic groups; 145–162 ppm, aromatic C–O groups; 162–190 ppm, COO/CON groups; 190–120 ppm, carbonyl groups. Integration was performed according to these ranges for the spectra obtained from different NMR techniques as shown in the case of IHSS and Minnesota HAs (Fig. 2). T_1-corrections were applied for HAs with incompletely-relaxed DP/MAS spectra. Below, the results of DP/MAS or corrected DP/MAS (both referred to a DP/MAS later for convenience) will be used as the standards for comparison because both techniques generate quantitative results when compared to elemental analysis [33]. Furthermore, if CP/MAS resonance frequencies are not specified, they are 75 MHz for ^{13}C.

As compared with DP/MAS (Fig. 2), ramp-CP/MAS displayed higher percentages for carbons between 0 and 108 ppm and low percentages between 108 and 220 ppm. CP/TOSS also had higher signal within 0–108 ppm and lower percentages of functional groups within 108–220 ppm. CP/MAS gave higher percentages for carbons between 0–108 ppm and 190–220 ppm, and lower percentages between 108 and 190 ppm. The signal between 0 and 96 ppm is from sp^3-C and between 108 and 220 ppm is due to the sp^2-C. The signal in the range of 96–108 ppm can be from both sp^2-C and sp^3-C.

Compared to CP/MAS, ramp-CP/MAS and CP/TOSS have lower intensity within the ranges of 0–108 and 190–200 ppm, and higher signal within the range of 108–190 ppm. These percentage are closer to the integration results of DP/MAS than to those of CP/MAS. There is not a clear trend for the comparison of ramp-

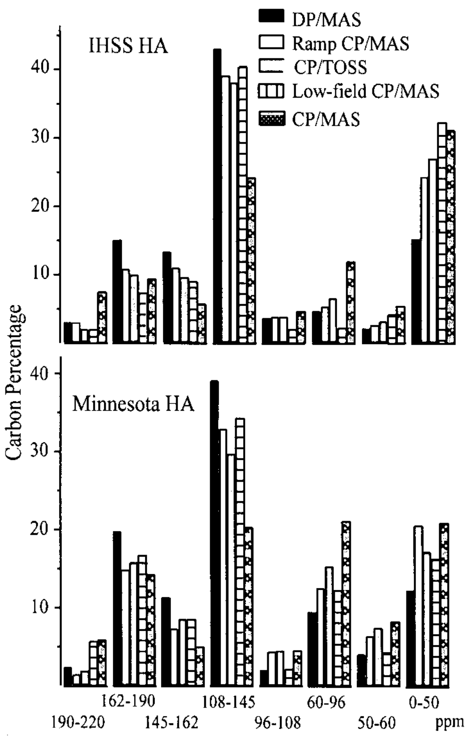

FIGURE 2 Integration results of different NMR techniques for IHSS and Minnesota HAs. Low fields are 50.3 MHz (^{13}C frequency) for IHSS HA and 22.6 MHz (^{13}C frequency) for Minnesota HA.

CP/MAS with CP/TOSS for the HAs tested and some integration regions are better for ramp-CP/MAS and others for CP/TOSS.

IHSS HA CP/MAS at the ^{13}C frequency of 50.3 MHz has better integration results than CP/MAS at the ^{13}C field of 75 MHz but not better than CP/TOSS or ramp-CP/MAS. The integration result of CP/MAS at the ^{13}C frequency of 22.6 MHz [41] was the closest one to its DP/MAS, better than ramp-CP/MAS, CP/TOSS and CP/MAS at the ^{13}C frequency of 75 MHz.

Figures 3–5 show the spectra of DP/MAS, ramp-CP/MAS, CP/TOSS, and 75 MHz CP/MAS of all the HAs; Fig. 5 also displays low-field CP/MAS of IHSS and Minnesota HAs [42]. DP/MAS, ramp-CP/MAS, and CP/TOSS displays quantitatively very similar spectral patterns for all HAs, with an almost identical number of peaks for a given HA (Figs. 3–5). However, the CP/MAS spectra exhibit more peaks than ramp-CP/MAS, CP/TOSS or DP/MAS because of spinning sidebands. In general, CP experiments have sharper peaks than DP because CP requires dipolar coupling between the proton and carbon nuclei and thus favors the detection of rigid regions, whereas DP can detect all kinds of carbons if a sufficient recycle delay time is allowed. The major advantage of CP is the better S/N.

The IHSS HA CP/MAS spectrum at the ^{13}C frequency of 50.3 MHz displays much larger sp^3-C and incorrectly lower carboxylic and aromatic C–O carbons than its DP/MAS spectrum as well as distinct sidebands (Fig. 5). It is just slightly better than its CP/MAS spectrum at the ^{13}C frequency of 75 MHz but worse than CP/TOSS and

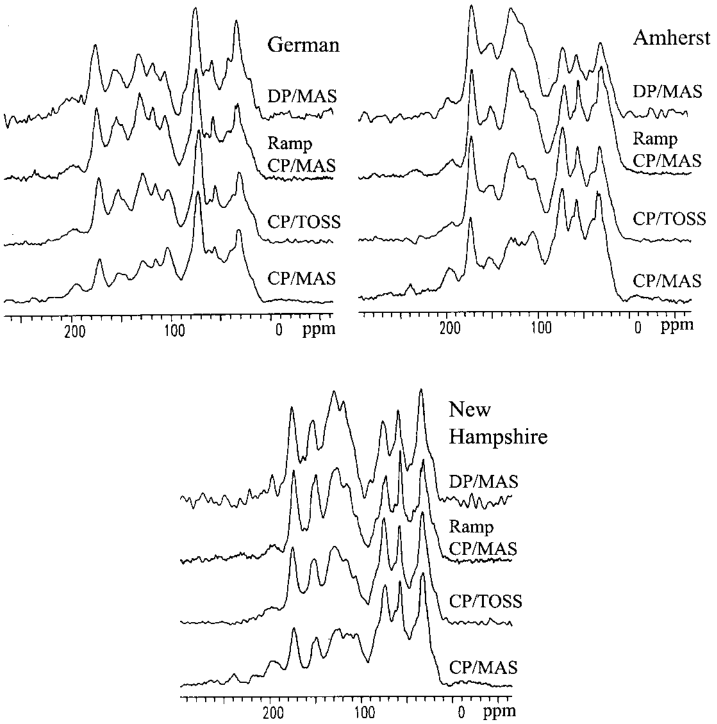

FIGURE 3 Comparison between DP/MAS (13 kHz spinning speed), ramp-CP/MAS (8 kHz spinning speed), CP/TOSS (4.5 kHz spinning speed), and CP/MAS (^{13}C frequency of 75 MHz and 5 kHz spinning speed) of German, Amherst and New Hampshire HAs.

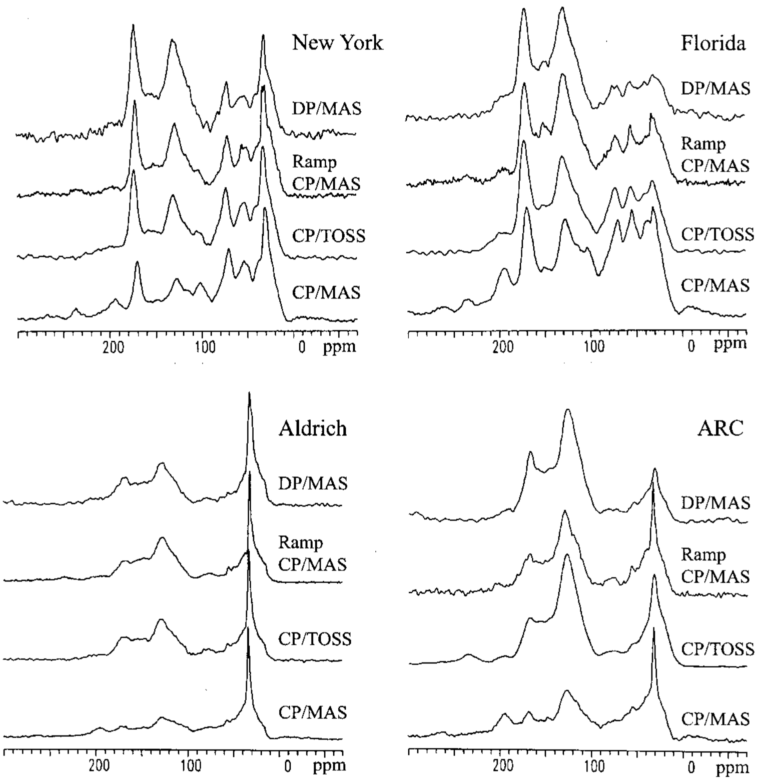

FIGURE 4 Comparison between DP/MAS (13 kHz spinning speed), ramp-CP/MAS (8 kHz spinning speed), CP/TOSS (4.5 kHz spinning speed), and CP/MAS (^{13}C frequency of 75 MHz and 5 kHz spinning speed) of New York, Florida, Aldrich and ARC HAs.

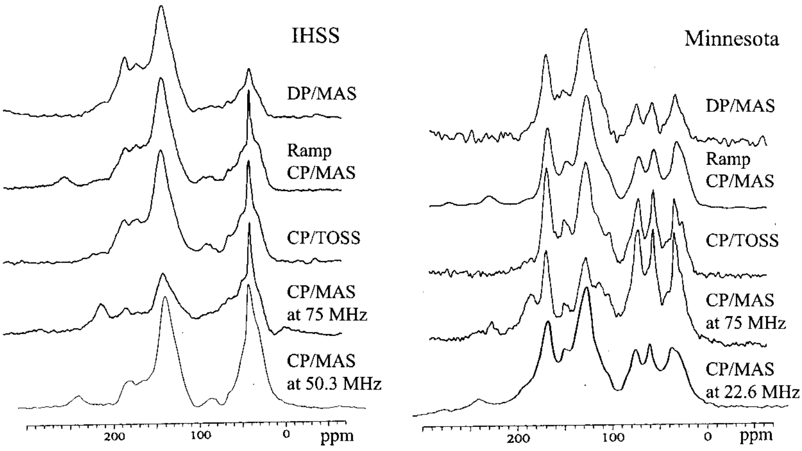

FIGURE 5 Comparison between ^{13}C MAS spectra of CP/MAS at low magnetic field (^{13}C frequency of 22.6 MHz and 4 kHz spinning speed and ^{13}C frequency of 50.3 MHz and 5 kHz spinning speed) DP/MAS (13 kHz spinning speed), ramp-CP/MAS (8 kHz spinning speed), CP/TOSS (4.5 kHz spinning speed) and CP/MAS (^{13}C frequency of 75 MHz and 5 kHz spinning speed) of IHSS and Minnesota HAs.

ramp-CP/MAS. The low magnetic field (50.3 MHz) leads to broader lines and lower resolution than the higher field (75 MHz). The Minnesota HA CP/MAS spectrum at the ^{13}C field of 22.6 MHz displays a pattern quite similar to its DP/MAS spectrum. However, it also has broader lines and lower resolution.

Standard CP/MAS

CP enhancement by means of the abundant ^{1}H spins can provide up to four times more ^{13}C signals than is obtained by DP/MAS NMR [40]. Furthermore, in the CP/MAS experiments the recycle delay between scans is determined by the longest T_1^{H} of the sample, not the longest T_1^{C} as for DP/MAS. Generally, T_1^{H} is shorter than T_1^{C} is a given sample. Therefore, in a given amount of time, many more CP/MAS experiments can be carried out. Nevertheless, as mentioned in the Introduction, several factors (low CP efficiency of unprotonated or mobile carbons; spinning sidebands; and baseline distortions) make CP/MAS spectra non-quantitative. Spinning sidebands produce particularly obvious distortions, especially in spectra acquired at a 75 MHz ^{13}C frequency (Figs. 3–5). CP/MAS overestimates the sp^{3}-C contents and underestimates the sp^{2}-C except for the carbonyl-C (Fig. 2). The sp^{2} carbons have a larger chemical shift anisotropy and therefore larger sidebands than sp^{3} carbons. As a result, the centerband area of sp^{2}-C is reduced more than that of sp^{3}-C. At spinning rates below 11 kHz, some of the sidebands of aromatic carbons occur in the sp^{3}-C region, increasing the relative sp^{3}-C percentage. This is the other important reason why CP/MAS overestimates sp^{3}-C. The high percentage of carbonyl-C integrated from CP/MAS is actually a combination of carbonyl-C and the sidebands of aromatic-C. The reasons for nonquantitation of CP/MAS has been discussed in detail elsewhere [11,13,14,16,17,25].

A simple way to avoid the sidebands in CP is to run samples at low field NMR ($B_0 < 2.35$ T). The CP/MAS spectrum at a ^{13}C frequency of 50.3 MHz is better than CP/MAS at 75 MHz but worse than CP/TOSS or ramp-CP/MAS, and its integration result is inconsistent with the results of the other techniques. The integration results of CP/MAS at the ^{13}C frequency of 22.6 MHz are better than those of ramp-CP/MAS, CP/TOSS and CP/MAS at the ^{13}C frequency of 75 MHz, quite close to DP/MAS. However, although its shape is generally similar to DP/MAS spectrum, the low magnetic field led to low resolution. In general, low magnetic field result in low sensitivity, low resolution, more scans and long machine time [26]. Considering the modern trend to high-field NMR spectrometers today, it is actually difficult to have access to low magnetic field NMR instruments. Furthermore, most of the CP efficiency problems still exist at low fields.

TOSS Spectra

There are two advantages for TOSS over simple CP/MAS. The first is that a good TOSS can eliminate all the sidebands, so that the spectrum shows only the true peaks for a HA sample. The second is that the implementation of CP/TOSS can avoid baseline distortion arising from the deadtime. However, because of their larger chemical shift anisotropy, sp^{2} carbon signal is less completely refocused than sp^{3}-C during the TOSS sequence. Thus, CP/TOSS underestimated sp^{2}-C as compared to DP/MAS. On the other hand, because protonated carbons relax more quickly than unprotonated carbons as shown in the CP/T$_1$−TOSS, the T_2^{C} relaxation during the

TOSS sequence with its duration of two rotation periods or more tends to decrease the signal of protonated aliphatic carbons more strongly.

The comparison of CP/TOSS or CP/MAS with DP/MAS (Figs. 3–5) shows that CP/TOSS was consistently better than CP/MAS for both quantitative and qualitative characterization/analysis. Spinning sidebands of CP/MAS distort the pattern and area of NMR spectra, particularly in the sp^2 region and it was almost impossible to analyze, even qualitatively, a HA sample from a 5-kHz CP/MAS spectra at 300 MHz (Figs. 2 and 3). Though the spectra obtained using CP/TOSS are not absolutely quantitative, comparisons of different CP/TOSS spectra for samples from similar sources can be made assuming the same CP efficiencies for the similar samples. It is strongly recommended that CP/TOSS be used instead of CP/MAS for a ≥ 200 MHz spectrometer.

Ramp-CP/MAS

In the conventional CP/MAS experiments, proton and carbon field strengths are adjusted to meet the Hartmann–Hahn (HH) condition. At a low spinning speed, matching profiles centered on the HH condition are broad. However, if the spinning speed increases, the HH matching profiles split into a series of narrow matching bands separated by the rotor frequency. Compared to the matching sidebands, the CP rate at the matching centerband, corresponding to the exact HH condition, is slower. To establish and maintain an efficient matching condition under high-speed MAS, Metz *et al.* [27,28] introduced an amplitude ramp on either of the radio-frequency channels during the contact time. It substantially improved the performance of the CP experiments at high spinning speeds and long contact times. Compared with conventional CP, a linear ramp of amplitudes centered at a sideband and covering its entire width greatly increased the signal intensity at long contact times. Furthermore, the broadened matching profile permitted deviation from an exact Hartmann–Hahn matching condition. Cook *et al.* [30,31] applied this technique in the study of a fulvic acid (FA) and a HA and reported improved results over CP/MAS for the humic substances used.

However, it should be noted that the transfer rate in the ramped CP is smaller than in regular CP at lower spinning speeds. The reason is that the matching condition is fulfilled only during part of the contact time. There is no problem for model substances with long $T_{1\rho}^{H}$ relaxation times. However, for HAs the short $T_{1\rho}^{H}$ relaxation times of the order of a few milliseconds result in significant and often differential signal loss during long ramped CP periods. It may even render some carbon species with short $T_{1\rho}^{H}$ relaxation times invisible [29].

These effects make ramped CP non-quantitative and Cook *et al.* [30] had to determine the appropriate contact time empirically for each sample, using the solution ^{13}C NMR spectrum of the same HA as a "standard". The acquisition of their solution NMR spectrum took about 2–4 days [30]. Furthermore, several ramp-CP/MAS spectra need to be run to obtain the "right" contact time [30]. The contact time of the ramp-CP/MAS spectrum which was most similar to the "standard" solution NMR spectrum was chosen as the "right" contact time. Hence, the contact time determination procedure is time-consuming and quite arbitrary. Moreover, solution ^{13}C NMR spectra of HA samples are not guaranteed to be quantitative. Solution-state NMR has the following limitations for characterizing humic substances: (1) samples may not completely dissolve, (2) solvents can modify humic substances structurally and (3) humin cannot be dissolved at all in an aqueous phase. Thus, if the determination of the contact time for a ramp CP experiment is based on solution NMR spectra, the ramp CP spectra can be only as good as those from solution NMR. As pointed out previously, the results from this study show the inconsistency of the ramp technique (at a contact time = 2.5 ms) as compared to DP/MAS (Figs. 3–5). Actually, our study has shown that ramp-CP/MAS at a spinning speed of 8 kHz was not significantly better than CP/TOSS at 4.5 kHz. Even though the ramp-CP/MAS is better than CP/TOSS, the major reason for this difference is the spinning speed. In our view, optimized CP and ramped CP will be similar at the same spinning speed. The advantage of fast spinning is smaller sidebands and the disadvantage reduced sensitivity because a smaller rotor (4-mm diameter) and thus smaller sample amount have to be used to obtain a high spinning speed.

Generally, if a semi-quantitative spectrum is needed, a CP/TOSS spectrum at the highest attainable spinning speed with carefully set Hartmann–Hahn match is a good resort. However, if a quantitative spectrum is required, DP/MAS should be employed for samples with reasonably short T_1^C's (less than 5 s) whereas DP/MAS corrected CP/T$_1$–TOSS should be employed for samples with long T_1^C, relaxation times.

Correction Factors

Correction factors for ramp-CP/MAS, CP/TOSS and CP/MAS were calculated as follows: Each spectrum is normalized to total unit area and divided into eight regions. The area of a given region is compared with the area of the corresponding region in the DP/MAS spectrum. For example, based on DP/MAS, the correction factor of CP/TOSS for the region of 190–220 ppm is:

$$\text{Correction factor} = \text{DP/MAS peak area}(190 - 220\,\text{ppm})/\text{CP/TOSS peak} \times (190 - 220\,\text{ppm}). \tag{1}$$

For each region, the correction factors were averaged over the nine samples and average correction factors plus its halt width at the 95% confidence level are listed in Table I. These correction factors provide a match with the quantitative DP/MAS (Table I). for ramp-CP/MAS, CP/TOSS, and CP/MAS, the correction factors for the regions of 0–108 ppm are smaller than 1 and for 108–220 ppm larger than 1, except for the 190–220 ppm region of the CP/MAS spectrum. This indicates that all the CP

TABLE I Correction factors for ramp-CP/MAS, CP/TOSS and CP/MAS[a]

ppm	190–220	162–190	145–162	108–145	96–108	60–96	50–60	0–50
DP/MAS	1	1	1	1	1	1	1	1
Ramp-	1.22[b]	1.35	1.24	1.13	0.79	0.76	0.83	0.74
CP/MAS	±0.38[c]	±0.05	±0.14	±0.08	±0.40	±0.11	±0.12	±0.13
CP/TOSS	1.13	1.29	1.39	1.27	0.78	0.63	0.72	0.74
$\nu_r = 4.5$ kHz	±0.30	±0.09	±0.14	±0.12	±0.40	±0.01	±0.10	±0.11
CP/MAS	0.53	1.53	2.03	1.74	0.66	0.47	0.58	0.67
$\nu_r = 5$ kHz	±0.19	±0.08	±0.26	±0.14	±0.34	±0.09	±0.10	±0.11

[a] Correction factors = peak area from DP/MAS peak area from ramp/CP/MAS, CP/TOSS or CP/MAS.

[b] Average of nine HAs.

[c] at 95% confidence level based on *t*-test.

techniques underestimate the sp^2 region and overestimate the sp^3 region, except for the carbonyl region of CP/MAS which is overestimated due to the overlap with sidebands of the aromatic carbons. By employing these correction factors, the deviation of the CP techniques from quantitation can be corrected. For "difficult" samples (low carbon%), CP/TOSS at the highest spinning speed possible for a large rotor (7-mm diameter) should be used and the peak areas obtained should be multiplied with the correction factors from Table I in order to obtain quantitative composition information.

CONCLUSIONS

Five solid-state ^{13}C NMR techniques have been compared in this study, with DP/MAS and corrected DP/MAS as quantitative references. All the techniques involving cross polarization are not quantitative. Although it can provide high sensitivity, CP/MAS at 300 MHz or higher frequency is good neither for quantitative nor for semiquantitive analyses of HAs due to sidebands and differential CP efficiencies. Instead, CP/TOSS is highly recommended at 300 MHz because it not only provides clear spectra but also displays better semi-quantitative results than CP/MAS. Ramp-CP/MAS does not provide significantly better results than CP/TOSS at the same spinning speed. We suggest that for characterization of HAs, before the application of any other NMR techniques, a simple CP/TOSS should be run to obtain clear qualitative information, which can be made at least semi-quantitative based on the Table I in this work, if similar acquisition conditions to ours are adopted. Low-filed CP/MAS (^{13}C frequency of 22.6 MHz) can provide better integration results than CP experiments at high field (^{13}C frequency of 75 or 50.6 MHz) but has lower sensitivity and may lose some structural resolution.

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